Received at: 1:?3PM, 20/9/2001
20.Sep. 2001 14:14 EPA MUENCHEN 089/2399-4465TION EATY Nr.6674 S. 1

S PILKINGTON
COPY JSM

by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MILES, John S.
ERIC POTTER CLARKSON
Park View House
58 The Ropewalk
Nottingham, Nottinghamshire NG1 5DD
GRANDE BRETAGNE

#0044-115 355 2201

COMUS | PARTNER JSM
NOTTM 20 SEP 2001
ACTIONED BY: SP

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	25.09.2001
REPLY DUE	**within 1 month(s)** from the above date of mailing

Applicant's or agent's file reference
ICOY/P23294PC

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03196	18/08/2000	20/08/1999

International Patent Classification (IPC) or both national classification and IPC
C12N15/62

Applicant
IMPERIAL COLLEGE INNOVATIONS LIMITED et al.

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain document cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 20/12/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Celler, J

Formalities officer (incl. extension of time limits)
Neumann, M
Telephone No. +49 89 2399 7351

WRITTEN OPINION

International application No. PCT/GB00/03196

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-100 as originally filed

Claims, No.:

1-47 as originally filed

Drawings, sheets:

1/17-17/17 as originally filed

Sequence listing part of the description, pages:

1-9, filed with the letter of 11.10.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

WRITTEN OPINION International application No. PCT/GB00/03196

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-5(partly),6,7,8-16(partly),17-24,25-39(partly),40,41,42-45(partly),46,47,

because:

   ☒ the said international application, or the said claims Nos. 1-5,8-16,36(IA) relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 47 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-5(partly),6,7,8-16(partly),17-24,25-39(partly),40,41,42-45(partly),46,47.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

WRITTEN OPINION International application No. PCT/GB00/03196

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

☐ all parts.

☒ the parts relating to claims Nos. 1-5(partly),8-16(partly,25-39(partly),42-45(partly).

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	2-4,12-15,25,30,32,34,36-39(NO)
Inventive step (IS)	Claims	1,5,8-11,16,26-29,31,33,35,42-45(NO)
Industrial applicability (IA)	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

WRITTEN OPINION
SEPARATE SHEET

International application No. PCT/GB00/03196

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Due to the lack of unity of the present international application (see International Search Report and Re Item IV), the ISR was established only for the subject matter of Claims 1 - 5 (partly), 8 - 16 (partly), 25 - 39 (partly), 42 - 45 (partly) and 47 (partly). Therefore, the present written opinion is formulated only in respect of said claims.

2. Claim 47 is so unclear (Art. 6 PCT) that formulation of a meaningful opinion on novelty, inventive step and the industrial applicability of the subject matter for which protection is sought was not possible. The subject matter has to be clearly defined in claims. Said claim does not identify any technical feature of the invention that would allow a skilled person to distinguish the method, use, etc. of the claim from any other method, use, etc. already known in the art. Furthermore, it si not clear to which part of the application the expression "as herein disclosed" refers to. As according to the provisions of Art. 6 PCT, the invention must be clearly defined in claims, said reference offends that requirement.

3. Claims 1 - 5, 8 - 16 and 36 relate to subject matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject matter of these claims(Article 34(4)(a)(i) PCT).
   For the assessment of the present Claims 1 - 5, 8 - 16 and 36 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

WRITTEN OPINION
SEPARATE SHEET

International application No. PCT/GB00/03196

Re Item IV

Lack of unity of invention

The present application refers to four inventions (see the International Search Report).

The first-mentioned invention is the subject matter of Claims 1 - 5 (partly), 8 - 16 (partly), 25 - 39 (partly), 42 - 45 (partly) and 47 (partly), which are directed towards forms of SNARE molecules resistant to cleavage by clostridial toxins, their uses and polynucleotides coding therefor.

The second invention is the subject matter of Claims 1 - 18 (partly), 25 - 39 (partly) and 42 - 47 (partly), which are directed towards the forms of SNARE molecules capable of blocking the proteolytic activity of clostridial toxins, their uses and polynucleotides coding therefor.

The third invention is the subject matter of Claims 25 - 28 (partly), 30 - 39 (partly), 42 - 44 (partly) and 46 - 47 (partly), which are directed towards forms of SNARE molecules capable of inhibiting SNARE-mediated exocytosis, their uses and polynucleotides coding therefor.

Finally, the fourth invention is the subject matter of Claims 26 - 28 (partly), 30 (partly), 33 - 35 (partly), 37 - 39 (partly), 40, 41, 42 - 44 (partly) and 47 (partly), which are directed towards a gene therapy delivery system based on proteolytically inactive form of clostridial toxin.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present Written Opinion is given only in regard to those parts of the international application that relate to what has been indicated in Re Item IV as the first invention i.e. the method of treating a patient suffering from poisoning or at risk of poisoning by clostridial toxin, use of SNARE in treatment of such a

patient as above, a method of the inhibition of exocytosis in a cell, all based on forms of **SNARE molecules resistant to cleavage by clostridial toxins**, said molecules themselves and polynucleotides coding therefor, the method of making such SNARE molecules and corresponding expression constructs, including gene therapy constructs and pharmaceutical compositions as well as kits.

2. Reference is made to the following documents:

D1: GONELLE-GISPERT C., HALBAN, P. A., NIEMANN H., PALMER M., CATSICAS S., SADOUL K.: 'SNAP-25a and -25b isoforms are both expressed in insulin-secreting cells and can function in insulin secretion.' BIOCHEMISTRY JOURNAL, vol. 339, 1 April 1999 (1999-04-01), pages 159-165, XP002159203

D2: SADOUL K., BERGER A., NIEMANN H., WELLER U., ROCHE P.A., KLIP A., TRIMBLE W.S., REGAZZI R., CATSICAS S., HALBAN P.A:: 'SNAP-23 is not cleaved by botulinum neurotoxin E and can replace SNAP-25 in the process of insulin secretion' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 52, 26 December 1997 (1997-12-26), pages 33023-33027, XP002159204

D3: BRUNSD D., ENGERS S., YANG C., OSSIG R., JEROMIN A., JAHN R.: 'Inhibition of transmiter release correlated with the proteolytic activity of tetanus toxin and botulinum toxin A in individual cultured synapsys of Hirudo medicinalis' JOURNAL OF NEUROSCIENCE, vol. 17, no. 6, 15 March 1997 (1997-03-15), pages 1898-1910, XP002159205

D4: WO 95 32738 A (ALERGEN) 7 December 1995 (1995-12-07) cited in the application

WRITTEN OPINION
SEPARATE SHEET

International application No. PCT/GB00/03196

3.1 D1 discloses SNAP-25 isoforms that have been made resistant to BoNT/E cleavage by means of sight-directed mutagenesis (e.g. p.163, "Production of BoNT/E-resistant SNAP-25 isoforms"). To obtain the toxin-resistant, mutant isoforms the sequence around the BoNT/E cleavage site was changed (e.g. p. 159, "Abstract"). The generated isoforms were transfected into cells prior to treatment with the toxin (e.g. p. 162, "Functional role of SNAP-25 isoforms of insulin secretion"). The mutants did not prevent the toxin cleavage of the endogenous SNAP-25, therefore were not inhibitory towards the endopeptidase activity of the toxin. However. they were able to **functionally replace** in exocytosis the endogenous, wild-type SNAP-25. D2 discloses a similar, functional replacement of the endogenous SNAP-25 with naturally occurring form of SNAP-23 which is not cleavable by BoNT/E. Consequently, a method of reversing the inhibition of exocytosis according to Claims 3, 4,12 and 36 is known from the prior art and not new in the sense of Art 33(2) PCT. Furthermore, as mutant SNAP-25 of D1 is a variant of SNAP-25 it also falls under the scope of Claim 30 and renders it not novel.

3.2 The mutant SNAP-25 of D1 or SNAP-23 of D2 also appear to be suitable for use in medicine or for use in manufacture of medicament for the treatment of a patient suffering from poisoning or at risk of poisoning by clostridial toxin, which, in turn renders the subject matter of Claims 2, 13 - 15, 25 and 32 not new (Art. 33(2) PCT). D1 discloses also the existence of the nucleic acid encoding the mutant SNAP-25 (e.g. p.160, "Mutagenesis of SNAP-25 isoforms"), which renders Claims 34, 37, 38 and 39 not new (Art. 33(2) PCT).

4.1 From D3 a skilled person would know that tetanus toxin proteolyses synaptobrevin and that synaptobrevin, SNAP-25 and syntaxin are targets of clostridial toxins (e.g. p. 1898, "Abstract" and "Introduction" as well as references therein). Furthermore, it is demonstrated in D3 that the amino acid residues on both sides of the toxin cleavage site in SNARES are responsible for the degree of cleavability and that the toxin resistant forms of these molecules can be created (e.g. p. 1902, "Leech SNAP-25 is resistant to cleavage by BoNT/A" also Fig. 2B). For example, particular importance of residue Q197 of mammalian SNAP-25 is made evident (Fig. 2B). The cleavage resistant forms of SNARE molecules were demonstrated to be able to render the synaptic transmission toxin insensitive (e.g. p. 1908, right-

hand-side column). The method of restoring SNARE based vesicle fusion of D3 has all of the essential technical features of the method of treatment of Claim 1. The difference between the method of Claim 1 and the disclosure of D3, seen as the closest prior art, lies in that the latter does not explicitly proclaim the method of treatment of a patient. However, In light of the information in D2 a skilled person faced with the problem of treating botulinum or tetanus toxin poisoning would see as an obvious choice for candidate treatment the toxin resistant forms of SNARE molecules. In consequence, the method of treatment of Claim 1 is not regarded as inventive in the sense of Art. 33(3) PCT. From D3 It is also obvious that the SNARE could be syntaxin or synaptobrevin. Therefore, Claim 5 also lacks the presence of inventive step.

4.2 Furthermore, the choice of residues surrounding the toxin cleavage site becomes obvious in light of D3. The importance for toxin resistance of the residues located in the proximity to the cleavage site is further underscored by D2 (e.g. p. 33024 "SNAP-23 is resistant to cleavage by BoNT/E"), which renders Claims 10, 11, 29, 31 and 33 not inventive (Art. 33(3) PCT). Also the fact that tetanus and botulinum toxin A , B, C1 as well as others can be involved appears obvious. In consequence Claims 8 and 9 lack inventive step.

4.3 D3 teaches a skilled person that specific forms of SNARE molecules are resistant to some, bu not all, specific types of toxins (e.g. p. 19088, right-hand-side column). In light of that, the need for identification of the type of toxin from poisoning with which a patient is suffering becomes a matter of obviousness, which renders Claims 16 and 45 not inventive (Art. 33(3) PCT).

4.4 Moreover, as the subject matter of Claim 30 is not new and that of Claims 29 and 31 is not inventive, the method of making a polypeptide according to Claim 35 also lacks inventive step (Art. 33(3) PCT).

4.5 D4 discloses (active or inactive) clostridial toxin, which is able to enter the target nerve cell and facilitate the introduction of a drug or any other biologically active molecule (e.g. p. 2, "Summery of the invention"). In light of the teaching of D4 It is obvious to use any inactive form of the toxin as the targeting moiety to bring any molecule attached to it into a cell that can be entered by the toxin moiety.

WRITTEN OPINION
SEPARATE SHEET

The approach of D4, seen as the closest prior art, differs from the subject matter of Claims 26, 27, 28 and 42 in that the part attached to the targeting moiety derived from the toxin, is a form of SNARE molecule or a gene therapy delivery systems. The forms of SNARE molecules and gene therapy delivery systems are however particular embodiments of the general approach of D4. Consequently, as the SNARE molecules of Claim 26 are not new (see above) and a gene therapy construct of Claim 39 is not new (see above) the subject matter of Claims 26, 27, 28 and 42 falls in its entirety under the scope of teaching of D4 and therefore lacks the presence of inventive step. Further introduction of technical features well known in the art, such as cell-specific promoter or a pharmaceutically acceptable carriers does not render the subject matter of Claims 43 and 44 inventive (Art. 33(3) PCT).

Re Item VII
Certain defects in the international application

According to the requirements of Rule 10.2 PCT, the terminology and the signs shall be consistent throughout the application. This requirement is not met in view, for example, of the use of three letter abbreviations for amino acids in Claim 10. For the same feature in Claim 11 single letter code is used.

Re Item VIII
Certain observations on the international application

1. The use of the expression "said" clostridial toxin in independent Claim 2 renders the subject matter unclear, because the clostridial toxin in the independent Claim 2 is not mentioned prior to that point. Therefore it appears that a reference is made to the subject matter disclosed outside of the claims which, in turn, would offend Art. 6 PCT.

2. Art. 6 PCT requires that the invention be defined in claims. The use of the expression "for example" in Claims 11, 29, 31 and 45 offends that requirement,

because it implies that the scope of protection sought embraces also embodiments that lie outside of the scope of the definition presented in the claims. To identify preferred embodiments, instead of "for example", "preferably" should be used.

3. The expressions "variant", "fragment" and "derivative" in Claims 30 and 37 render the scope of the subject matte unclear (Art. 6 PCT). It is not clear to a skilled person which and how many residues of the molecule can be changed and/or deleted or added as well as what other modification can be carried out so that the molecule is still regarded as a "variant", "fragment" or "derivative" of SNARE. For example, from an extreme view point an oligonucleotide 2- or 3-nucleotide long or peptide 2- or 3-amino acid long, can be regarded also as a "variant", "fragment" or "derivative" of SNARE. In consequence there is a large number of molecules known from the prior art which foll under the scope of said claims which makes acknowledgement of novelty not possible.

The applicant is invited to file new claims which take account of the above comments. In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, no matter whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based (see also Rule 66.8(a) PCT).
If the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed.
Any information the applicant may wish to submit concerning the subject-matter of the invention, for example further details of its advantages or of the problem it solves, and for which there is no basis in the application as filed, should be confined to the letter of reply and not be incorporated into the application (Article 34(2)(b) PCT).